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Computational Design and Evaluation of ScFv-IFN-β Fusion Protein against HER1-positive Breast Cancer

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ABSTRACT

OBJECTIVE: Human epidermal growth factor receptor 1 (HER1)-positive breast cancer, is characterized by HER1 overexpression that promotes aggressive tumor development and treatment resistance. The objective of this study is to computationally design immunocytokines capable of targeting cancer cell antigens and modulating the immune response, representing a promising therapeutic approach for HER1-positive breast cancer

METHODOLOGY: This is an in silico-based study, in which a novel immunocytokine was engineered by linking an anti-EGFR/HER1 single-chain variable fragment with interferon-β. In this design, the ScFv moiety targets HER1-positive tumor cells, while the IFN-β domain retains its natural specificity for IFNAR1/IFNAR2 and is intended to provide localized immunemodulatory signaling at the tumor site. Sequence data for the ScFv and for IFN-β were concatenated to yield the ScFv-IFN-β design. Physicochemical metrics including molecular weight, extinction coefficient, and instability index were calculated via ProtParam and SOLpro. The 3D conformation was predicted using AlphaFold, followed by refinement with GalaxyRefine. Structural quality was assessed using PROCHECK and RAMPAGE. Potential allergenic sites were mapped using AlgPred, and the corresponding mRNA secondary structure was analyzed with RNAfold. Protein-receptor interactions were examined through docking on the HDOCK server, and molecular dynamics were simulated on the iMOD platform. The study was conducted at the National Center of Excellence in Molecular Biology, University of The Punjab, Lahore. This study was carried out between 2021-2022.

RESULTS: The fused protein displayed acceptable physicochemical characteristics, retained a plausible and well-folded conformation, maintained a robust mRNA secondary structure, and demonstrated favorable docking poses for both HER1 and interferon receptors. Stability metrics from the dynamic simulations corroborated these findings.

CONCLUSION: The ScFv–IFN-β fusion protein emerges as a theoretically interesting design for targeted therapy against HER1-positive breast cancer, but the evidence at this stage is purely computational.

KEYWORDS: Breast cancer, Fusion protein, Human Epidermal Growth Factor Receptor 1 (HER1), Interferon Beta (IFN-β), immunocytokine, Single-chain Variable Fragment (ScFv).

INTRODUCTION

Breast cancer ranks as the second-highest cause of cancer-related mortality among women across the globe, right behind lung cancer, as noted by Afzal, et al (1). Clinicians routinely grapple with the tumors' vast cellular diversity, or heterogeneity. Standard protocols-chemotherapy, radiotherapy, and surgery-tend to work best while the disease is still confined to localized sites; once it spreads, their impact often dwindles. During earlier stages, some patients discover that treatments are ineffective or cause more harm. Decision regarding care is usually based on the patients' genetic and epigenetic makeup (2). This reliance highlights a growing demand for therapies that step outside the traditional pathways. Researchers have begun exploring next-generation agents such as immunotoxins, cytokine-chimeric fusions, and tumor-necrosis-factor-linked proteins, with experimental results appearing in various literature (3). One of the promising methods involves immunocytokines, hybrid molecules that combine antibody-targeting precision to cytokine-driven immune boosting (4). Zhang et al (5), demonstrated that immunocytokines can adapt to different tumor microenvironments. That adaptive behavior stems from the dual action built into each immunocytokine: one end binds a specific tumor antigen while the other end sparks local immune activity. Early preclinical data summarized by Mobark, Hull (6) show that characteristic keeps leukocytes focused on the malignancy yet spares surrounding healthy tissue. Such tailor-made delivery could help oncologists customize therapy on a case-by-case basis rather than leaning on broad population averages.

Gout, Groen (7) point to a growing clinical interest in the promise of immunocytokines to inflict a customized punch on breast cancers. The HER1 receptor, immobilized in the plasma membrane, is a tyrosine kinase, the activity of which is strongly correlated with the growth and development of certain breast tumors. In about 15 to 30 percent of cases, especially in triple-negative subtypes, overexpression of HER1 is followed by brisk tumor progression and shrinking therapeutic opportunities. EGF and TGF- activate HER1, releasing PI3K/AKT and MAPK/ERK cascades that feed cell proliferation and motility.

Antibody constructs as well as small-molecule tyrosine kinase inhibitors now dot the clinic, demonstrating substantial benefit to HER1-positive patients. Yet, clinical experience reveals an unsettling truth: tumors often retool their signaling architecture and sidestep these targeted approaches.

Recombinant antibody engineering has recently pivoted toward a novel class of fusion proteins that combine the selective binding of an antibody fragment with the direct immune signaling of a cytokine. Versions of this concept are commonly grouped under the label immunocytokines. In the prototype molecule, IFN- and a recombinant single-chain variable fragment (ScFv)-built from the dispersed variable-heavy and variable-light domains-handle the cytotoxic and targeting tasks in tandem (5, 8-9). By concentrating both effectors at the tumor interface, the chimeric design boosts local cell-killing activity while sparing healthy tissues from the widespread inflammatory surge typical of free cytokines (10). Among the catalogue of possible immune signals, type I interferons-whether - or -retain special prominence because they simultaneously provoke T-cell mobilization, activate dendritic and macrophage guardians, and curtail aberrant blood vessel sprouting (3,4). IFN-β further distinguishes itself by dampening the auto-growth circuits of neoplastic cells, inhibiting telomerase function, silencing c-myc transcription, and priming the TRAIL pathway that drives tumor-cell suicide (9).

The origin of the immunocytokine concept stretches back to the late 1990s, but clinical momentum dissipated more than a decade later, long enough for skepticism to crystallize. Nonetheless,

recent modeling studies hint that fusing a single-chain variable fragment with interferons can narrow the therapeutic window without flattening it entirely (10). Greater design flexibility in the ScFv moiety lets researchers swap, truncate, or extend masking and targeting domains, so prototype molecules resemble less a rigid framework and more a modular kit (11). Toward that end, the current investigation fuses a HER1-directed ScFv domain to IFN-β so that the antibody fragment localizes the construct to HER1-positive breast cancer cells, while IFN-β can exert its effects through its cognate interferon receptors (IFNAR1/IFNAR2) in the same microenvironment. In silico docking is therefore used to explore plausible engagement of the fusion with HER1 and interferon receptors, with the understanding that IFN-β itself does not bind HER1.

Study Rationale

Despite the development of HER1-targeted therapies, the treatment of HER1-positive breast cancer remains difficult because of limited selectivity, off-target toxicity, and immune evasion. Immunocytokines chimeric proteins of a tumor-targeting antibody fragment and an immune-stimulating cytokine have the potential to resolve the issue by localizing therapeutic activity at the tumor with the activation of anti-tumor immunity. The targeting of an anti-HER1 single-chain variable fragment (ScFv) with interferon-β (IFN-β) may localize the therapy at the tumor site in a better way, giving double receptor-targeted and immunomodulatory activity, and minimize systemic adverse effects.

METHODOLOGY

Study Design

This in silico study describes the computational design and characterization of a novel immunocytokine (ScFv-IFN-β) generated by fusing an anti-EGFR/HER1 single-chain variable fragment (ScFv) with human interferon-β (IFN-β). The work was carried out at the Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan, during the period 2021–2022. All molecular modeling, sequence optimization, structure prediction, physicochemical analysis, docking simulations, and immunogenicity/allergenicity predictions were performed using publicly available bioinformatics tools and servers. No experimental wet-lab validation was conducted in this study.

Sequence Analysis and In Silico Construct Design

The amino acid strings for interferon- (IFN-β) and single-chain variable fragment (ScFv) were extracted from GenBank under accession numbers ABS89222.1 and AFF61391.1. In silico assembly of the ScFv domain involved concatenating the light-chain and heavy-chain variable regions with the flexible linker SSGGGGSGGGGSGGS. To connect the finished ScFv to IFN-β compound, a second linker, GGGGSGGGGSGGGGS, was inserted; previous work shows such extensions improve folding and maintain structural integrity (12). A 6-His tag (HHHHHH) was appended to the N-terminus of the entire fusion for straightforward chromatographic purification.

Predicting Physicochemical Properties

A suite of standard physicochemical parameters was computed to gauge how the immunocytokine behaves in solution and interacts with cellular environments. These include estimated half-life, molecular weight, net charge, instability index, theoretical isoelectric point, aliphatic index, amino-acid profile, and the grand average of hydropathicity. For the calculations, ExPASy ProtParam provided a familiar workbench (13).

Solubility itself was modeled using SOLpro, a web-based platform that relies on a trained ensemble of machine-learning predictors. The system has earned empirical backing in several wet-lab comparisons, making it a favored choice when sole sequence data are available (14). Predictive solubility is reported in natural log units of milligrams per milliliter.

Tertiary Structure Prediction, Refinement, and Validation

AlphaFold (alphafold.ebi.ac.uk) delivered the initial three-dimensional model, a choice driven by its recent reputation for producing protein structures that rival experimental data in terms of fidelity (11). To further refine the conformation, the top-scoring model was then run on the GalaxyRefine server that optimizes atomic contact with molecular dynamics-like procedures. Final stereochemical integrity was confirmed with the RAMPAGE as well as PROCHECK web servers, both of which highlight the torsion-angle distribution as well as the geometrical clashes within the chimeric construct (15-16).

Prediction of mRNA Stability

Detection of the secondary fold of an RNA chain can be indicative of biochemical functions hidden within ostensibly quiet sequences. The RNAfold web service (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi), calculates such structures based on minimizing free energy and provides a quick snapshot of potential base-pairings for the transcript of the immunocytokine.

Molecular Docking

Molecular docking investigations typically depend on computational power to start with binding guesses, and the HDOCK web server-http://hdock.phys.hust.edu.cn-fills that gap by merging the

template-free with the template-guided strategies. The server has itself been evaluated with various test sets and can therefore generate orientation predictions that still retain statistical significance. Without using the docking score as a direct surrogate for binding affinity, the result shows how a ligand can fit into its receiving binding site even under the default binding orientations, enabling quick, qualitative comparisons of docking poses between many complexes. As a result, rather than being quantitative estimates of binding free energy, the docking results are interpreted as relative pose preferences and interface patterns. The current exercise included six different combinations: the HER1 receptor with the solo ScFv; the receptor with the ScFv-IFN-β fused construct; the fusible module against either IFN R1 or IFN R2; and lastly, free IFN-β against both receptor chains. Each protein construct was represented in PDB format and submitted in one go, because treating them as a set simplifies the writing of the post-docking script and the checking of the alignment.

Molecular-Dynamics Simulation

Docked protein structures were uploaded to the iMODs portal (https://imods.iqfr.csic.es/) for in-depth Molecular Dynamics Simulation. The web-based platform records how the torsional angles of the interface evolve overtime. Built-in Normal Mode Analysis quantifies each assembly's flexibility and stability while keeping processing costs manageable. From the same run it extracts root-mean-square deviation profiles, eigenvalue distributions, covariance maps between residues, and local deformation patterns. Those metrics collectively flag regions of structural rigidity and slack, then summarize interatomic coherence. For this study, default parameters were simply retained after submission, minimizing the need for manual tuning.

RESULTS

Chimeric Protein Construction

Single-chain variable fragment and interferon-beta sequences were fused via a 15-residue linker to yield the ScFv- IFN-β construct. The antibody fragment contributes 242 amino acids, with a second linker of 15 units uniting the light-chain and heavy-chain variable-domains. For translational stability, an N-terminal extension of methionine-glycine-serine-serine and a 6-histidine affinity tag were appended. The interferon-beta moiety contains 167 amino acids, so the entire chimeric protein measures 434 residues. A schematic representation of the arrangement is provided in supplementary figure 1.

Physicochemical Properties and Solubility

ProtParam and the SOLpro provided a summary of the protein's baseline characteristics. ScFv-IFN- β weighed roughly 48,173 Da, a figure consistent with similar fusions. Because -3.777 appears in the GRAVY calculation, the construct is expected to prefer aqueous environments rather than membrane-like solvents. The aliphatic index of 73.38 suggests that a substantial fraction of its side chains is hydrophobic. A theoretical isoelectric point of 9.02 combined with a net charge of plus five indicates that the molecule will reside in the positive region of a standard pH gradient. A solubility score of 0.634 (probability threshold >0.5) indicates that the ScFv-IFN construct is predicted to be soluble, but this remains a probabilistic in silico estimate and not a guarantee of solubility across all experimental buffers.

3D Model Building, Refinement, and Validation

The AlphaFold web service produced four separate models of fused protein as well as individual ScFv, IFN-β , ScFv- IFN-β and HER1 architectures; these representations are shown in figure 1 (A-D). Each construct was assigned a confidence score, and the single model accruing the highest c-score was retained for downstream evaluation.

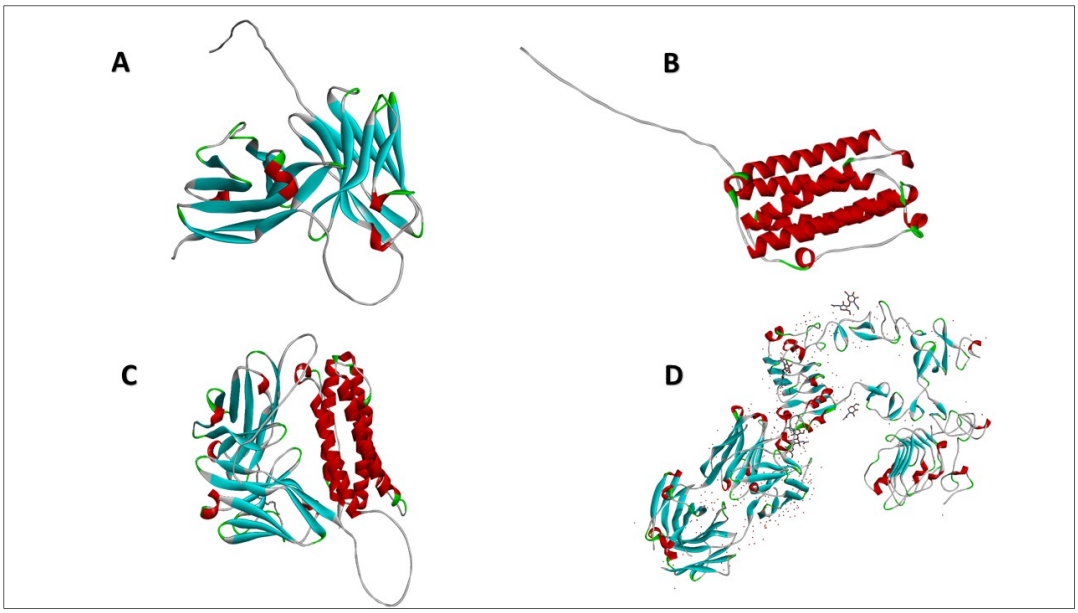

Figure 1: AlphaFold's 3D structural predictions of single proteins.

(A) ScFv structure illustrates immunoglobulin fold with well-defined VL and VH domains united by a flexible linker with the support of its antibody-like binding function.

(B) IFN-β has a helical cytokine structure required for receptor binding and for signal transduction downstream.
The ScFv-IFN-β fusion protein illustrates spatial separation of antibody and cytokine domains by the inter-domain linker, which prevents steric hindrances and allows for independent functioning of the domains.
The structural architecture of the HER1 receptor is depicted symbolizing its docking surface. The predicted structures were used as a reference point for the assessment of protein-protein interactions and stability by docking and dynamics calculations.
The AlphaFold predictions provided per-residue pLDDT confidence scores, with most residues in the structured domains falling in the high-confidence range (pLDDT > 70), indicating generally reliable backbone placement in these regions, whereas flexible loops and linker segments showed lower confidence values, consistent with increased structural uncertainty.To enhance the structure further, the initial 3D coordinate set was submitted to the GalaxyRefine, which subsequently proposed five distinct refinements. Of those five candidates, the single model that displayed the greatest accumulation of favored conformations in Ramachandran space was ultimately selected, as illustrated in Figure 2. Model quality assessment was conducted via PROCHECK and RAMPAGE, enabling a systematic comparison between the pre-refinement and post-refinement data.

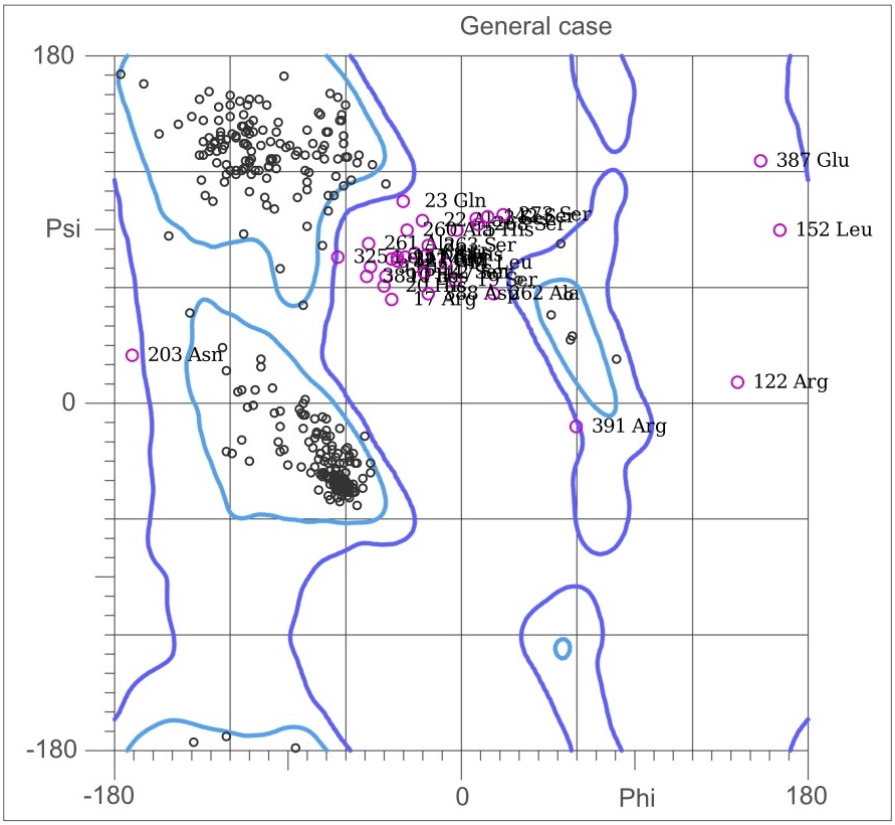

Figure 2: Ramachandran plot of refined 3D model of ScFv-IFN-β fusion protein.

Allergenicity predictions

Allergenicity predictions were generated using the AlgPred web portal, yielding a score of -0.74675496. Since scores below the default threshold of -0.4 indicate a probable non-allergen, the chimeric protein is predicted to be non-allergenic by this in silico method. However, a negative AlgPred score only suggests low allergenicity risk and does not constitute definitive proof of safety. Final confirmation of non-allergenic status requires experimental testing, such as in vitro IgE-binding assays or appropriate clinical studies.

mRNA folding stability

The strongly negative ΔG value indicates a thermodynamically stable predicted mRNA secondary structure, but does not by itself guarantee efficient expression; actual expression levels would need to be assessed experimentally.

Molecular docking

Protein-Protein docking was carried out on six protein-protein complexes, and the individual arrangements were grouped accordingly. For each configuration, scoring metrics such as the absolute docking score, the confidence score, and the ligand root-mean-square deviation, measured in angstroms, were recorded-a summary appears in Table 1. As shown in panels A and B of Figure 4, the designed ScFv–interferon–β fusion protein generated one of the favorable docking poses toward the epidermal growth factor receptor 1 (HER1). However, given the high ligand RMSD values (45–86 Å), these docking models are interpreted as qualitative representations of potential binding interfaces rather than evidence of strong, well-defined binding affinity. In contrast, IFN-β alone showed significantly lower RMSD values (~1 Å) with IFN receptors, indicating more stable and reproducible binding orientations. Residues Q65, S147, A151, D236, Y238, G251, K254, A262, G277, N368, and H371 formed multiple hydrogen-bond contacts with HER1 residues in the top-ranked HER1–ScFv–IFN–β pose, suggesting a plausible interaction interface. However, because of the high ligand RMSD values (45–86 Å), these models are interpreted only as qualitative representations of potential contact regions and not as evidence of strong or well-defined binding affinity

Table I: Molecular docking of protein complexes

No.	Docked complexes	Docking score	Confidence score	Ligand RMSD (Å)
1	HER1 receptor vs ScFv	-259	0.89	45.89
2	HER1 receptor vs ScFv-IFN-β	-285.3	0.94	53.23
3	ScFv-IFN-β vs IFNR1	-255.9	0.89	86.07
4	ScFv-IFN-β vs IFNR2	-255.5	0.89	64.31
5	IFN-β vs IFNR1	-390.7	0.99	1.03
6	IFN-β vs IFNR2	-456	0.99	0.6

Abbreviations. HER1; Human Epidermal Receptor 1, ScFv; Single Chain Fragment Variable, INF-β; Interferon beta, IFNR; Interferon Receptor

Molecular dynamics simulation

The simulation model displayed a steady increase in flexibility across the amino acid sequence, underscored by an eigenvalue of 1.747149 10-6, a numeric indicator of low mechanical stiffness. Heat-map correlations coupled with the observed low root-mean-square deviation confirmed that many side chains were strongly interacting with one another (shown in Figure 5). Figure 6 assembles key results from iMODS molecular-dynamics analysis: panel A plots the temperature-

factor distribution, panel B tracks local deformability, and panels C through F present eigenvalue dispersion, variance color-coding, covariance mapping, and a network elasticity scheme, respectively.

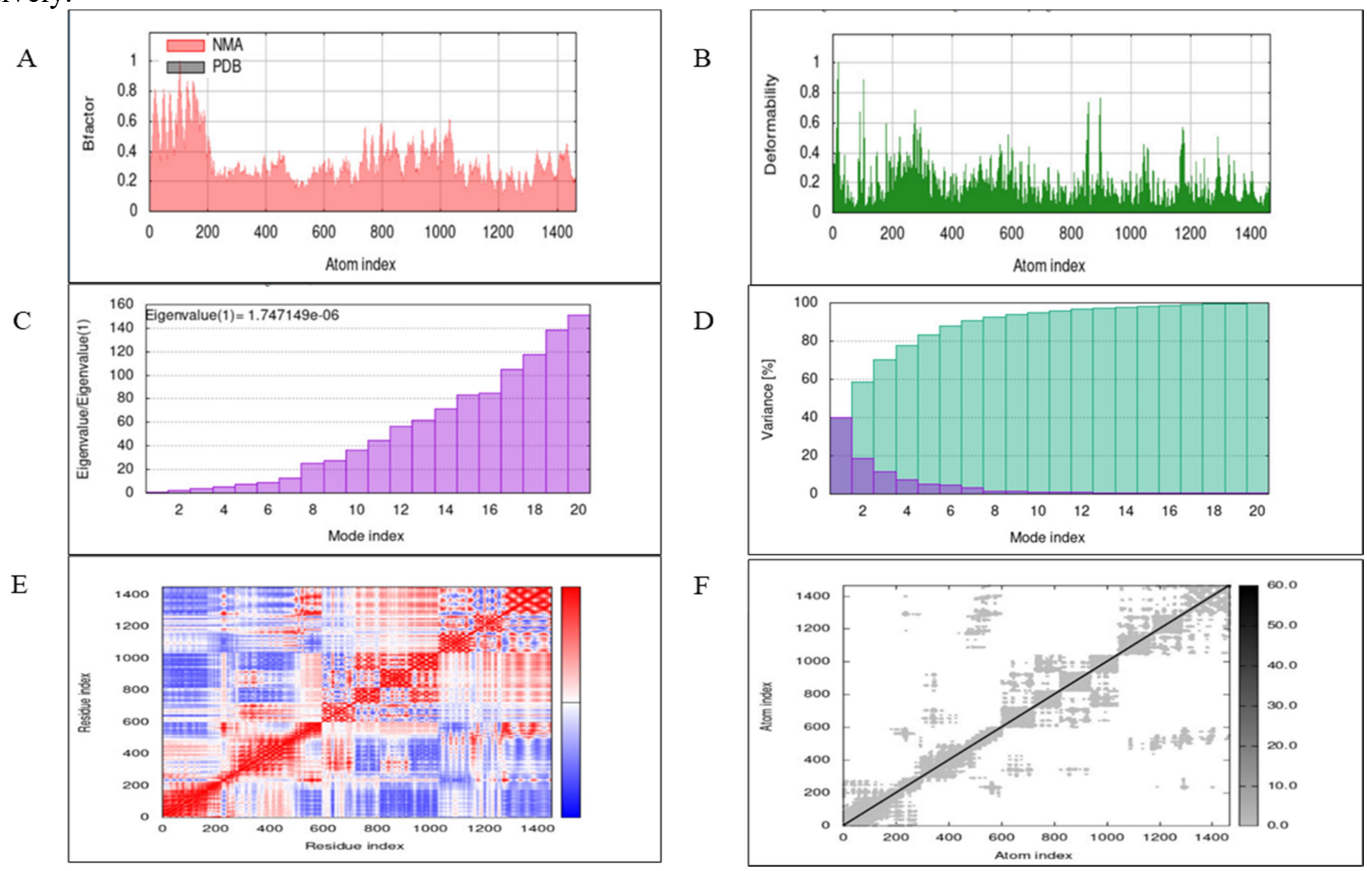

Figure 5: The results of molecular dynamic simulation study of ScFv-IFN-β and HER1 receptor docked complex

Results of molecular dynamics simulation of ScFv-IFN-β and HER1 receptor docked complex in iMODs.

(A) B-factor plot indicates thermal motion, low values indicating stable regions of binding.

(B) Deformability analysis reveals few peaks above residues, indicating minimal flexibility and a rigid, stable interaction.

(C) Eigenvalue of 1.747149e-06 reflects low stiffness, i.e., the complex can dissipate dynamic interactions without sacrificing structural integrity. The plot of variance is correlated, which is typical for a stable docked state. (E) Covariance matrix confirms high residue correlation of residues that are interacting with one another, indicating coordinated motion on interaction. (F) Elastic network model (shaded areas = stiffness) reveals extremely few high-stiffness areas, again demonstrating the structural rigidity of the complex.

DISCUSSION

The global incidence of breast cancer continues to rise, a trend most observers blame partly on the lack of highly selective treatment protocols that are also gentle on normal tissues (17, 18). Researchers remain troubled by the disease's unrelenting high mortality and morbidity, facts that continue to pressure the laboratory to refine existing therapies and create new ones that truly enhance survival prospects. They still rely on surgery, radiotherapy, and chemotherapy today; but each of them carries old familiar baggage-recurrence, cruel side effects, and in some settings, infuriatingly brief prolongations of life (19, 20). Targeted immunotherapy has begun to carve out a different narrative, and early reports suggest it frequently outperforms the older strategies when the tumor microenvironment allows (21). A sub-category attracting attention is the immunocytokine, a chimeric molecule that can home in on multiple tumor-associated antigens such as carbonic anhydrase IX, EGFR, EpCAM, FAP, and splice variants of fibronectin and tenascin (22). This poly-specificity may be especially advantageous for breast-cancer patients whose tumors exhibit marked amplification of the HER1 receptor.

The present study engineered an immunocytokine by pairing a HER1-directed single-chain variable fragment with an interferon-β coding sequence, thereby aiming to amplify the oncolytic response. Comparable constructs have outperformed standard systemic therapies in numerous preclinical models and are now under scrutiny in first-in-human trials (23).

Single-chain fragments have repeatedly proven stable and fold quickly into functional conformations, even after overnight incubation at elevated temperatures (10). Their modest molecular weight promotes rapid intra-tumoral distribution while limiting unintentional engagement of Fc receptors on macrophages, dendritic cells, and both T-and B-cell populations.

Interestingly, one-domain scaffolds such as nanobodies or fibronectin domains achieve their tertiary structure with minimal post-translational refinement, a striking contrast to the complex glycosylation and multi-sheet folding of conventional IgGs (10). Interferon- is not resting on its laurels; when a cell senses a virus or simply overheats, it releases this cytokine as an early warning flag (24). Receptor docking studies always show that binds more strongly than the partner, a feature reproduced by tighter immunological grip (25). Clinical optimism is moderated, however, as in the bloodstream the protein has a half-life of under 90 minutes and can become hypersensitive to normal tissue, side effects that dampen its anticancer activity (25). Recent technological developments have stretched that short half-life to approximately 15 hours in adult immunocytokines, at least giving physicians an even opportunity at scheduling effective doses (24, 25). Attachment of the cytokine to one-chain antibody variable fragment from an HER1-targeting antibody generates a guided missile: the payload targets, adheres, and only then releases its immune storm (26). Once inside a tumor, Interferon holds the cellular clock still at S phase and quietly silences the c-myc switchboard, preventing runaway growth (27). By waving the Fas and TRAIL flags, it sets off the caspase-8 execution pathway, effectively marking diseased cells for demolition (28). Even in trace amounts, the cytokine throttles new blood vessel construction, starving tumors of nutrients.

Research conducted in vitro suggests that the cytotoxic impact of IFN-β-coupled immunocytokines is primarily attributable to interferon activity itself, rather than to any antibody component (29). Parallel in vivo experiments reveal that an ErbB2-targeting formulation of IFN-β can completely stall the growth of humanized-mouse gastric-cancer xenografts, an outcome linked to the mobilization of the host immune system (30).

A different set of experiments led by Chan Gyu Lee and colleagues noted favorable therapeutic responses when IFN-β was paired with trastuzumab, based on trials performed across several cancer-cell lines (25). In an independent project, Asma Vafadar and coworkers engineered a recombinant single-chain variable fragment (ScFv) fused to the Cj-CdtB toxin and confirmed that the ScFv remains a stable polypeptide with strong binding to the HER2 receptor (31). Parnian Navabi and associates pursued a separate immunocytokine strategy, linking the same ScFv to an IL-2Rα module and demonstrating robust HER2 engagement as well as successful expression in the periplasmic space of E. coli BL21 (DE3) (32). Our own research confirms these results with demonstration of an analogous, favorable interaction between ScFv-IFN-β and the HER1 receptor.

Molecular docking generated a favorable docking score for the ScFv-IFN-β chimeric protein complexed with the HER1 extracellular domain (score = -285.3; confidence score = 0.94). However, this score cannot be interpreted as a quantitative binding affinity measurement. The high ligand RMSD value (53.23 Å) indicates substantial pose variability and limits confidence in the predicted interface. For comparison, IFN-β alone produced even more favorable scores with IFN receptors (–390.7 and –456) but achieved significantly lower RMSD values (~1 Å), reflecting more stable and reproducible docking poses. Evidence supporting that prediction is enhanced by a docking consensus measured better than 0.94 over replicate runs.

The fusion protein also generated docking poses with interferon receptors IFNR1 and IFNR2; however, these complexes exhibited even higher RMSD values (64–86 Å), making them unsuitable for structural interpretation. The computational data thus support the feasibility of the fusion engaging multiple receptors, but do not constitute evidence of superior dual targeting compared to the individual components. Quantitative analysis of the HER1 pose disclosed an unexpected group of hydrogen bonds linking receptor sidechains to fragment residues; such hooks tend to be related to higher specificity and kinetic stability in cellular contexts. If the in-silico image holds up against cellular assays, then immunocytokine ought to differentially label HER1-overexpressing breast carcinoma because it alone spares most normal epithelia. Binding affinity is only half the story; within the same molecule the yeast-derived ScFv component supplies antigen selectivity and the interferon payload supply anti-proliferative and immune-modulating activity to the same general area. Personalized medicine uses are immediate, especially for those patients already no longer benefiting from conventional monoclonal antibodies or small-molecule TKIs. Although the current findings remain proof-of-concept, they justify rapid progression into complementary in vitro and rodent xenograft studies prior to any clinical heuristic.

The research examined a suite of characteristics for the recombinant ScFv-IFN-β fusion protein: physicochemical stability, solubility at physiological salts, structural integrity, potential allergenicity, mRNA longevity in vitro, and binding affinity to target receptors. Polypeptide sequences of IFN-β and the antibody-derived ScFv were retrieved from the NCBI GeneBank databases under accession numbers ABS89222.1 and AFF61391.1 respectively. A flexible SSGGGGSGGGGSGGS linker stitched together the variable light and heavy chain domains before a second GGGGSGGGGSGGGGS extension fused the fused ScFv to the cytokine to create the ScFv- IFN-β construct. Previously published reports indicate that both the total length and specific residue arrangement of linker segments can dramatically influence the proper folding and thermal stability of ScFv scaffolds (31, 33). The predominance of small hydrophobic residues, primarily glycine and serine, confers an intrinsically flexible profile to the connector peptide without enforcing rigid secondary structures. For practical chromatography applications a

Gs4Hs3 tag was appended immediately upstream of the coding sequence to streamline purification and isolate the target fusion in near-native form.

The physicochemical properties of ScFv-IFN-β were checked according to ProtParam and SOLpro results. ProtParam measures a variety of physicochemical parameters, including molecular weight, isoelectric point, extinction coefficient, instability index, and grand average of hydropathicity. SOLpro provides information about protein solubility, given the amino acid sequence. Recent studies show the importance of designing a stable 3D structure and its effect on the molecular functions of chimeric proteins (34). AlphaFold was employed to predict the 3D structure of ScFv-IFN-β protein and GalaxyRefine was used to refine the structure. The structural integrity of the model was assessed using PROCHECK and RAMPAGE. AlgPred was used to predict the allergenicity and RNAfold was used to analyze ScFv-IFNβ mRNA stability. HDOCK was used for docking and to calculate the binding affinity ScFv-IFNβ with the HER1. While iMOD was used for protein-protein interaction and molecular dynamic simulations.

Immunocytokines have the potential to treat different cancer types. However, limited studies have been conducted on immunocytokines. The antigen expression and mutation status of cancer cells may possess a challenge to its effectiveness. This study aimed at in-silico studying and designing an effective immunocytokine showing favorable binding affinity for HER1. However, further research is needed to validate the effectiveness of immunocytokines, particularly the ScFv-IFNβ against HER1-positive breast cancer. Which will lead to development novel cancer therapeutics.

CONCLUSION

This research provides exploratory in-silico modeling of the ScFv-IFN-β fusion protein against HER1-overexpressing breast cancer; however, several important caveats must be emphasized. First, the high RMSD values for fusion protein–receptor complexes (45–86 Å) indicate unreliable docking convergence and prevent confident structural interpretation of binding interfaces. Second, the computational scores alone cannot be used to rank binding strength, as evidenced by the more favorable HDOCK scores for IFN-β alone compared to the fusion. Third, the work is purely theoretical, with biological efficacy, immunogenicity, and pharmacokinetics completely unproven in vitro or in vivo. In-vitro and in-vivo tests will need to confirm binding affinity, expression stability, and therapeutic effect. Second, variability in cancer heterogeneity and HER1 expression or mutation status might influence responsiveness, and therefore more detailed patient data analysis or personalized strategies may be required. Although accurate computational software such as HDOCK, AlphaFold, and RNAfold were utilized, cross-validation against other software or more up-to-date models such as RoseTTAFold or ChimeraX would increase prediction certainty. Subsequent research involves expression and purification of the construct in *E. coli* or mammalian cell systems, assessment of bioactivity in HER1-positive breast cancer cell lines, and in-vivo efficacy in xenograft models. Further investigation into potential immune-related adverse events or off-target effects is necessary.

Recommendations

Further in-vitro and in-vivo work is required to confirm the therapeutic activity, stability and safety of ScFv-IFN-β chimera. Furthermore, comparative analysis with existing therapeutic strategies should be conducted to evaluate the potential of ScFv-IFN-β chimera.

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